B cell responses to food allergy

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Abstract

Background: Understanding the mechanisms of tolerance induction to food allergens is very crucial for the development of medical treatments in food allergies. Several immune cells are involved during to food oral immunotherapy (Food-AIT), however, the role of allergen-specific B cells in the induction of allergen tolerance remains unclear. Therefore, the aim of this study is to demonstrate the role of allergen-specific B cells and compare the differences of gene expression profiling in allergic children during oral immunotherapy and natural tolerance induction.

Methods: Peripheral blood mononuclear cells (PBMC) from cow's milk allergic children, who followed the clinical food allergen-specific immunotherapy (Food-AIT) and who have developed natural tolerance were isolated. αS_1-casein-specific and non-specific B cells were identified and purified using dual-color staining with fluorescently labeled αS_1-casein allergen by flow cytometry. The immortalization of αS_1-casein specific B cells was performed through transduction with a retroviral vector containing GFP, BCL6, and Bcl-xL and expanded by culturing with CD40L and IL-21. Total and specific IgE, IgG and IgG subclass (IgG1 and IgG4) antibodies from culture supernatants of immortalized B cells were measured by ELISA. The single-cell/ Ultra Low RNA next-generation sequencing was performed for quantitative transcriptomics.

Results: After purification of αS_1-casein specific B cells and non-specific B cells, we measured the Ag-specific Ig profile to confirm their specificity. Specific IgE, IgG1, and IgG4 production from culture supernatants of αS_1-casein positive B cells were significantly elevated compared to αS_1-casein negative cells, while total IgE, IgG1, and IgG4 levels were comparable. The in-depth analysis of gene expression showed significantly different αS_1-casein-specific B cells of allergic children before and after OIT

compared to natural tolerance. The top 200 differentially expressed genes were shared between allergic children before and after OIT and natural tolerance. Within these shared significant genes, we identified roughly 30 tolerance-induced genes display similar gene expression patterns in allergic children after Food-AIT compared to natural tolerance. For examples, gut homing marker genes including CCR6 and CXCR5 are upregulated, immunoregulatory genes including IL10RB and IGHG4 are upregulated, and allergic asthma and atopic dermatitis-related genes including MAPK6, SMC6, DIMT1, CKAP2, AXL, and LHFPL2 are downregulated. After Food-AIT proinflammatory cytokine machinery was shut down and tolerance related genes were upregulated.

Conclusions: Our data suggest that allergen-specific B cells in food allergic children compared to natural tolerance display clearly different gene signatures. Approximately 30 tolerance-induced genes were identified. The in-depth analysis of significant genes are suggesting the cells migrate to gut with the presence of immunoreguatory molecules and regulate the allergic-related genes.

Keywords: allergen-specific B cells, food allergy, alpha S 1 casein, tolerance induction, gene expression.